CHEMISTRY OF NATURAL PRODUCTS

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This is to certify that the dissertation is the original contribution of the candidate and is suitable for submission for the partial fulfilment of the degree of Master of Philosophy.

Nizam-ud-Din Khan

(Nizamuddin Khan)
Supervisor

A C K N O W L E D G E M E N T

It gives me a great pleasure to express my deep sense of gratitude to Dr. Nizamullin Khan, whose consistent guidance and encouragement helped me in carrying out the work, presented in this dissertation.

I am highly grateful to Professor W.Rahman, Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh for providing necessary facilities.

I am highly indebted to my other laboratory colleagues for continuous help and encouragement.

Mehtab. Parveen

MEHTAB PARVEEN

C O N T E N T S

Part A

Part B

PART – A

INTRODUCTION

Cupressus, a member of sub-family Cupressoideae (family-cupressaceae), constitutes twenty species largely distributed in the Northern Hemisphere.[1]

Chemical Constituents of Cupressus

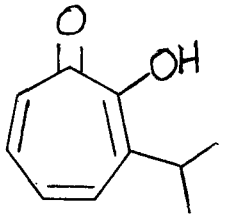
The compounds most frequently encountered in the genus, Cupressus are tropolones, terpenoids, and flavonoids. The scarcity of alkaloids in conifers is unfortunate since these compounds are physiologically important and also have taxonomic value.[2]

Tropolones

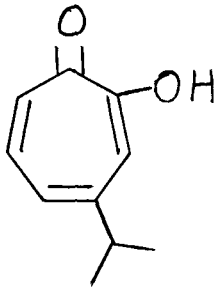
All tropolones so far isolated from Cupressus exhibit distinct structural relations to the terpenes. They differ in this respect from the tropolones produced by certain moulds and Colchicum species (meadow saffron).

The thujaplicins exhibited puzzling properties and appeared to be of aromatic nature somewhat resembling salicylaldehyde. However, they show no sign of carbonyl groups and were stronger acids (pKa 7) than normal monophenols. Furthermore, these compounds showed characteristic spectral properties.[3,4]

α-Thujaplicin(I), β-thujaplicin(II) and γ-thujaplicin (III) are typical constituents of Cupressus macnabiana.[5,6] Compounds I & II were also isolated from Cupressus macrocarpa[6] and C.torulosa.[7] The heartwood of C.arizonica[8] and C.stephensonii[10] constitute compounds II and III γ-Thujaplicin was also found in C.lusitanica[11] heartwood. β-Thujaplicin was isolated from C.abramsiana,[5] C.goveniana,[5] C.sargentii,[5] C.sempervirens[5] and C.pygmaea[9] as well.



(I)



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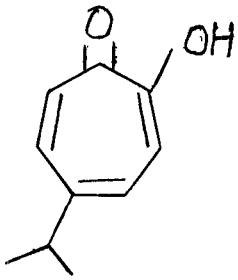
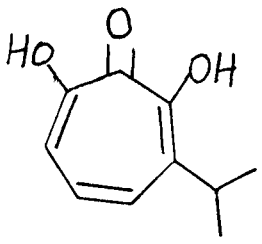


(III)

δ-Thujaplicinol(IV) occurs in the wood of C.abramsiana,[5] C.goveniana[5] and C.pygmaea,[9] while its isomer β-Thujaplicinol(V) is found in C.arizonica,[5] C.sargentii[5] and C.torulosa.[7]



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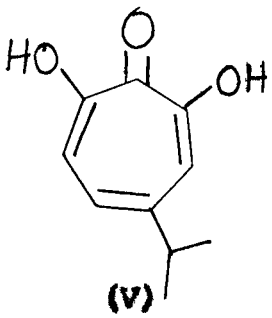
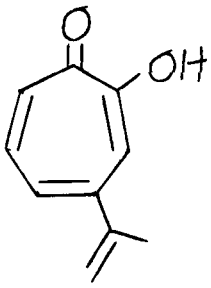


(V)

Dehydrothujaplicin-β-Dolabrin (VI) and β-Dolabrinol (VII) occur in C.abramsiana[5] C.goreniana[5] and C.pygmaea[9]. Later two species also contain, Pygmacin (VIII). β-Dolabrin (VI) has also been reported from C.sargentii[5]



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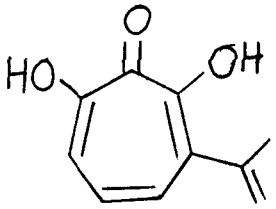

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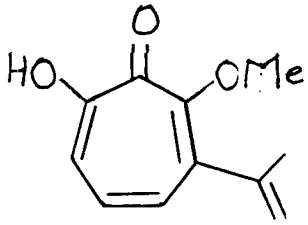
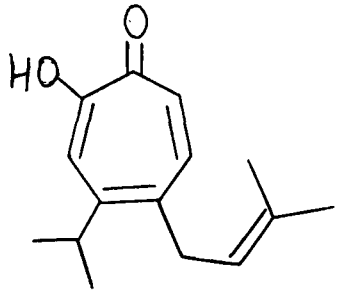


(VIII)

Nootkatin (IX), a sesquiterpene tropolone was isolated from C.arizonica[5,8] C.abramsiana[5] C.goreniana[5] C.macnabiana[13] C.macrocarpa[6] C.pygmaea[9] C.sargentii[5] C.sempervirens[5] C.torulosa[14] C.stephensonii[10] while its carbinol derivative, nootkatinol (X) occurs in C.lindleyi[38]



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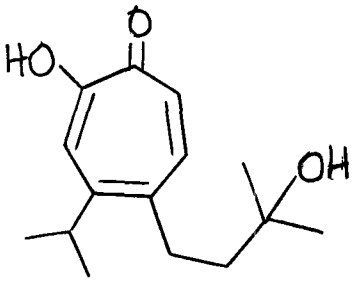
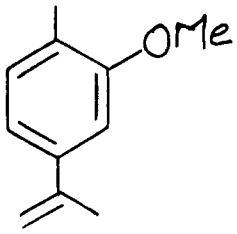


(X)

A C-10 compound with aromatic ring, Carvacrol methyl ether (XI) has been reported from C.arizonica[5,8] C.macnabiana[5,13] C.macrocarpa[6] C.torulosa[7] C.stephensonii[10] and C.sempervirens[5].



(XI)

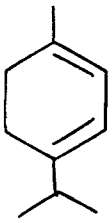
C.macnabiana shows a distinct behaviour in tropolone distribution compared to other Cupressus taxon.

Terpenes

Terpenes are the main constituents of the extractives of most woods of Cupressales.

Monoterpenes

The wood extractives of Cupressus abramsiana,[23] C.goveniana,[23] C.macrocarpa,[23] C.pygmaea[23] and C.sargentii[23] constitute α-terpinene (XII), γ-terpinene (XIII) and α-pinene (XIV), compounds XII & XIV also occur in C.lusitanica.[23]



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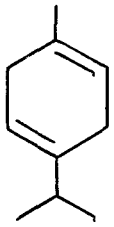

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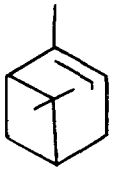

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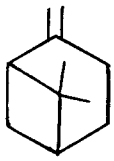
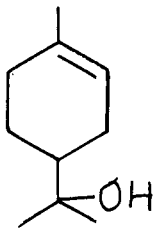


(XV)

α-pinene (XIV) has also been isolated from C.funebris,[24] C.stenhensonii,[10] C.sempervirens,[25] C.duprezianus,[26] β-pinene (XV) is relatively less common and was reported only from C.funebris,[24] C.macrocarpa[23] and C.sarmentii,[23] α-terpinol (XVI), p-cymene (XVII), Camphene (XVIII), Ocimene (XIX),



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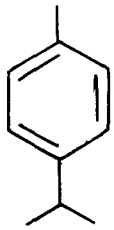

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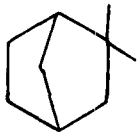

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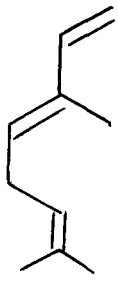

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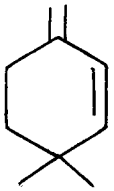

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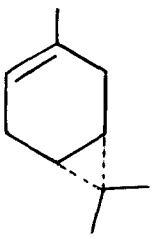
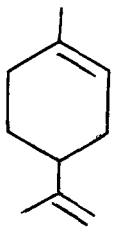


(XXII)

(XXIII)

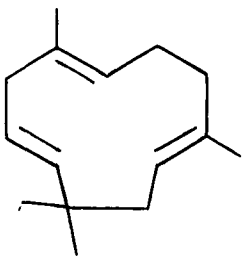


(XXI)

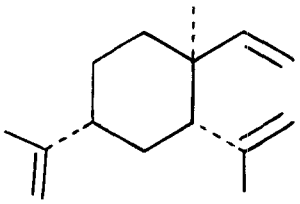
β-phellandrene (XX), Limonene (XXI) and ∝-thujene were isolated from C.lusitanica.[27] Occurance of compounds XVII, XVIII, XIX, XXI and myrcene (XXIII) in C.funebris[24] XVII, XVIII, XX, XXIII and sabinene in C.lusitanica[23]; XX to XXIII and sabinene in C.sargentii[23] XX, XXI and sabinene in C.abramsiana[23] and sabinene in C.goveniana,[23] C.macrocarpa[23] and C.pygmaea[23] has also been reported. 3-Carene occurs in C.goveniana[23] C.sempervirens[27], C.pygmaea[23] & C.lusitanica.[23]

Sesquiterpenes

Sesquiterpenes of the monocyclic bisabolane type were isolated from the few Cupressus species, for example, C.sempervirens[27] and C.lusitanica[28] (heartwood) were found to contain ∝-curcumene. The structure of the monocyclic sesquiterpene Humulene (XXIV), a constituent of C.arizonica[8] and C.torulosa,[7] containing an eleven membered ring has been firmly established by X-ray studies. β-Elemene (XXV), a sesquiterpene of Elemane type has been reported from C.sempervirens[27] and C.lusitanica.[27]

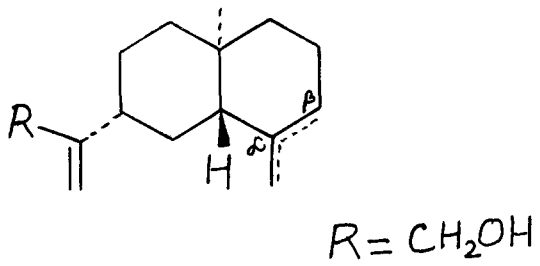


(XXIV)



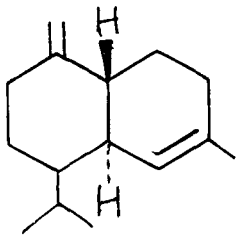
(XXV)

Sesquiterpenes of the selinane type and related compounds are the common Cupressus constituents, particularly, α- and β-selinenes were reported from C.sempervirens[27] and C.dupreziana[28] and Selinene-11-o-4β is also isolated from C.dupreziana[26] (heartwood). Costol (sesquibenihiol) (XXVI), an alcohol, was isolated from C.duprezziana[26] (heartwood).



(XXVI)

Cadinenes, Cadinols and related compounds are widely distributed in nature and usually occur as a mixture of considerable complexity. There has been much confusion in the chemistry of these compounds because of heterogeniety of fractions. Cadinene type terpenes are the common Cupressus constituents. Copaene (XXVII) has been isolated from Cupressus duprezziana[27] which contains δ-cadinene (XXVIII) as well.



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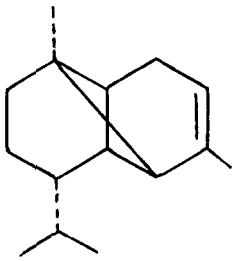
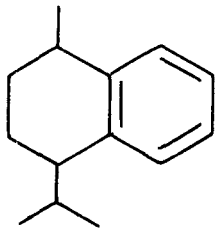


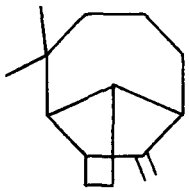
(XXVII)



(XXIX)

Calamene (XXIX) was found to occur in C.sempervirens[27] and C.lusoziana[27] only.

Tricyclic sesquiterpene longifolene (XXX) and Juniperol (XXXI) have been reported from C.lusoziana[27] heartwood and C.macrocarpa[15] bark.



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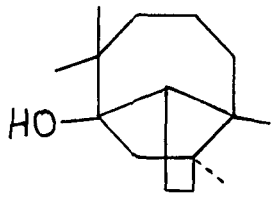
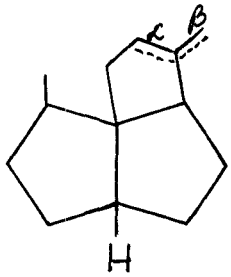


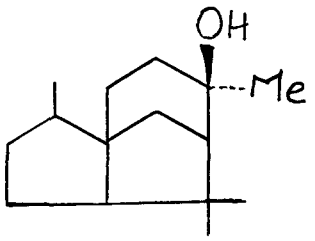
(XXXI)



(XXXII)

α - Cedrene (XXXII), a constituent of C.arizonica[8] heartwood is found to co-occur along with the 1,7-

diepi-β-cedrene and 1,7-diepi-α-cedrene in the heartwood of C.sempervirens[27] and C.dupreziana[27,28]. Diepi-1,7-cedrene-8 ol-15 and (8 βH) diepi-1,7-cedranol-15 were also isolated from C.dupreziana heartwood along with cedrol (XXXIII), a constituent of C.arizonica[8] and C.sempervirens.[16]



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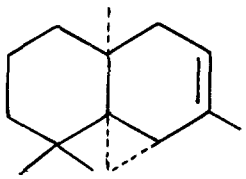
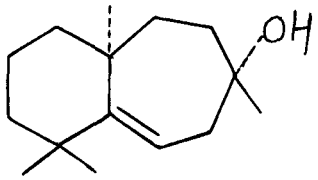


(XXXIV)



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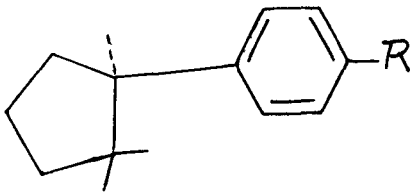
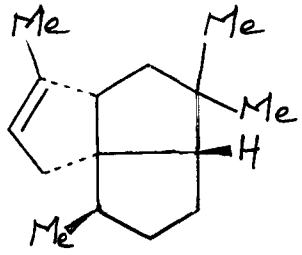


$R = CH_3$

(XXXVI)

Thujopsene (XXXIV), co-occuring with the widrol (XXXV) in C.arizonica,[8] has been reported from the heartwood of C.torulosa[7] and C.dupreziana.[27]. Later two species also contain cuparene (XXXVI), a constituent of C.sempervirens.[27]

C.dupreziana is unique in constituting several sesquiterpenes which are not common in other Dupreaeus species. α-Funebrine (XXXVII),[30] co-occuring with α-dupreziaenene (XXXVIII) in C.dupreziana,[30] has been reported only from C.funebris leaves.[29] Preizianone, α- and β-acoraliene has been isolated from C.sempervirens[27] and C.dupreziana.[27] Later species also contains γ-acoraliene. In addition to the above mentioned sesquiterpenes, borneol (XXXIX), Alaskaliene-4, 11-ol-14 (XL), Alaskaliene-4,7 ol-14 (XLI) and Alaskaliene-3,11 ol-14 have been reported from C.dupreziana.[26]



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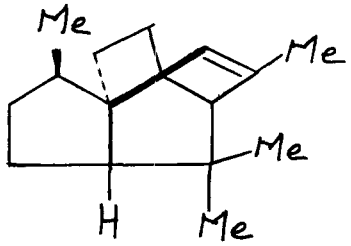

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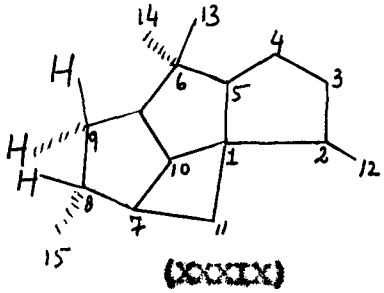

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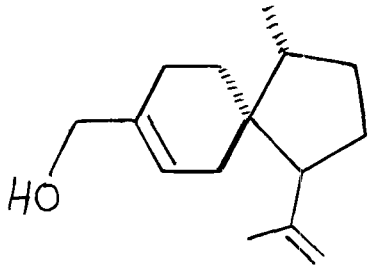
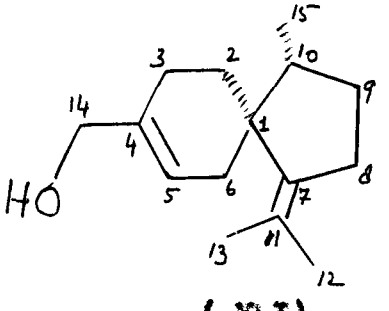
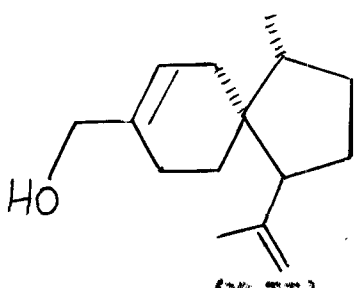


(XL)



(XLI)

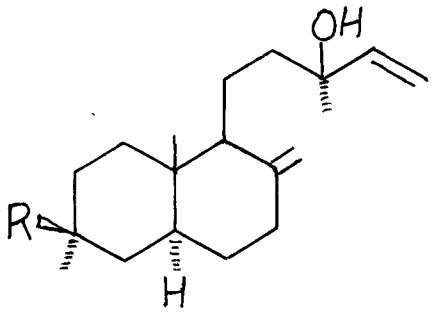


(XLII)

Diterpenes

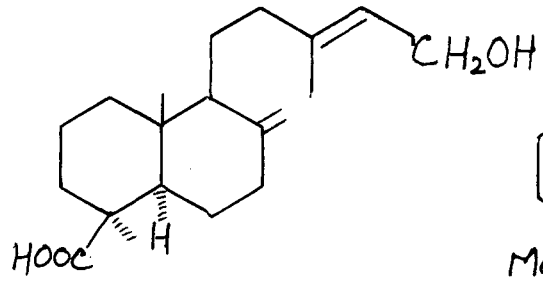
Several bicyclic, tricyclic and tetracyclic diterpenes have been reported from Cupressus species.

Bicyclic diterpenes with labdane skeleton, such as, Manool (XLIII), torulosal (XLIV) and torulosal (XLV) have been reported from Cupressus torulosa[7] and C.dupreziana[17]. Manool (XLIII) and torulosyl-acetate (XLVI) also occur in C.sempervirens[16] and C.dupreziana[17], respectively. C.torulosa[18] and C.sempervirens[19] also contain cupressic acid (XLVII). Neocupressic acid (XLVIII)

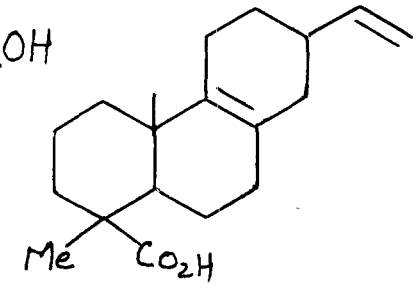


(XLIII)	manool	CH_3
(XLIV)	torulosal	CH_2OH
(XLV)	torulosal	CHO
(XLVI)	torulosyl-acetate	CH_2OAc
(XLVII)	Cupressic acid	COOH

and isocupressic acid (XLIX) have also been reported from the cones of *C.sempervirens*[20].

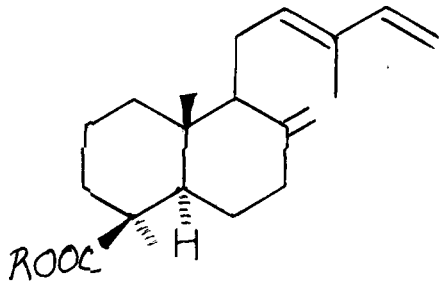


(XLIX)



(XLVIII)

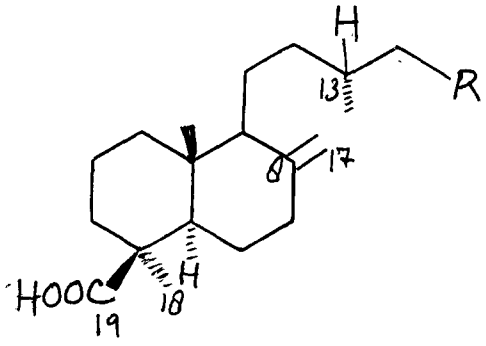
Labdatriene skeleton is represented by communic acid (L) in *C.torulosa*[21] and *C.sempervirens*[22]. *C.torulosa*[21] also contains methyl (+)-communate (LI) and (+)-semperviral. Labimes with saturated side chains,



(L) Communic acid R = H

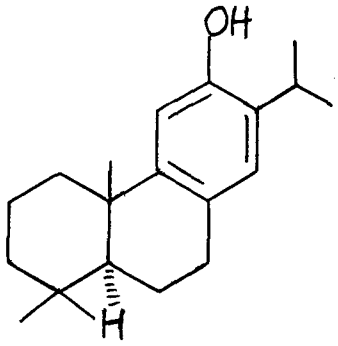
(LI) Methyl (+)-communate R = Me

imbricatolic acid (LII) and (+)-acetyl imbricatolic acid (LIII), are found to occur in *C.torulosa*[16].

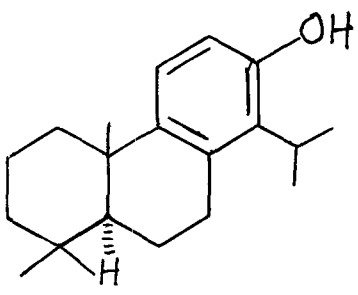


	R
(LII) imbricatolic acid	CH_2OH
(LIII) (+)-acetylimbricatolic acid	CH_2OAc

Several abietanes and structurally related pimaranes have been found in *Cupressus* species. Leaves & stem bark of *C.torulosa* contain ferruginol (LIV) and (+)-totarol (LV).

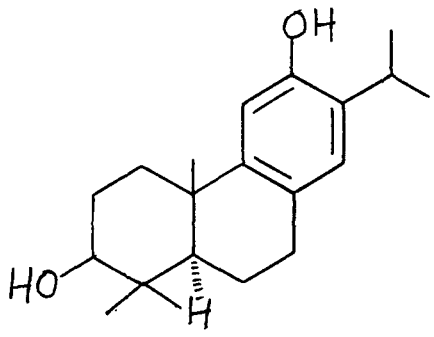


(LIV) Ferruginol

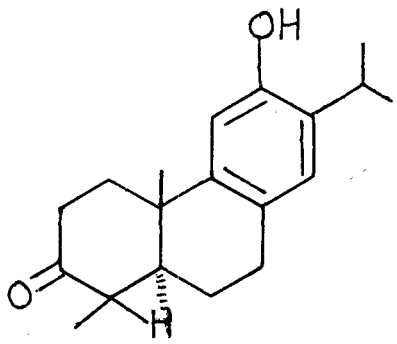


(LV) (+)-totarol.

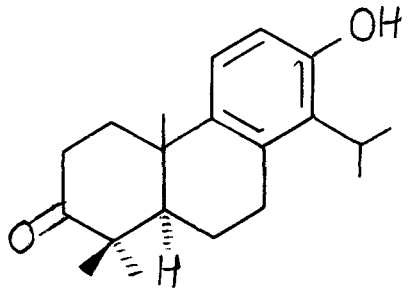
Two constituents of *C.torulosa*[7] heartwood, (+)-hinokiol (LVI) and (+)-hinokione (LVII), also occur in *C.lusitanica*[17] along with totarolone (LVIII), while diterpenes are different



(LVI) (+)-hinokiol



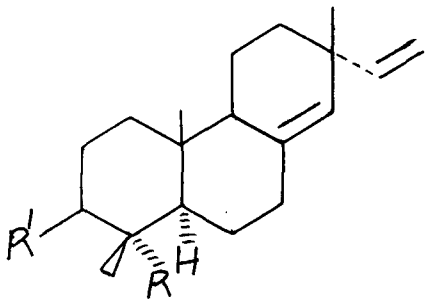
(LVII) (+)-hinokione



(LVIII) totarolone

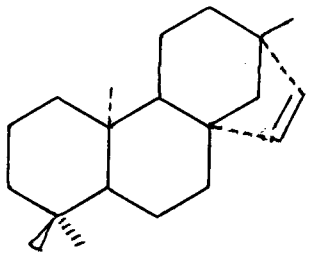
in these species. *C.torulosa*[18] constitute sandaracopi-

maric acid (LIX), while *C. dupreziana*[17] contains pimarinol and sandaracopimara-8, 15-diene-2β-ol. (LX).

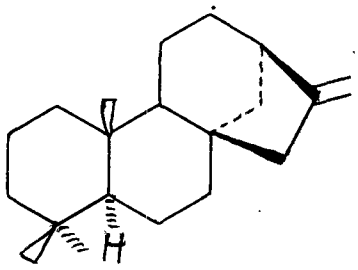


		R	R'
(LIX)	Sandaracopimaric acid	COOH	H
(LX)	Sandaracopimara-8, 15-diene-3β-ol	CH_3	OH

Tetracyclic diterpenes, (+)-hibaene (LXI) and (+)-phyllocladene (LXII) have been reported from *C. macrocarpa*[15].



(LXI)



(LXII)

Flavanoids:

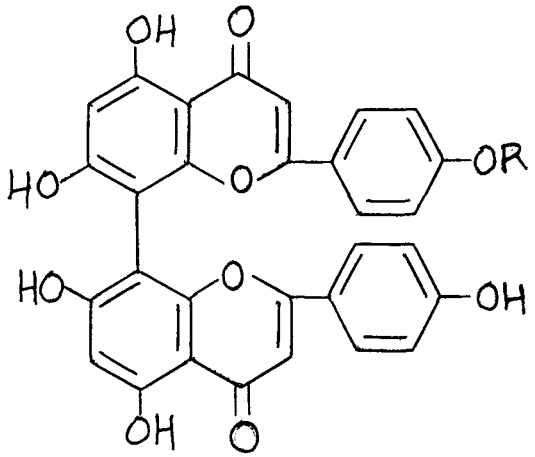
Flavanoids are found to co-occur alongwith procyanidin[18], catechin tannin and proanthocyanidin tannin[32] in C.torulosa. In C.goveniana[34] & C.australis[35] apigenin and its dimer are found to co-occur.

Cupressus Biflavonoids:

Biflavonoids with C-C and C-O-C link have been isolated from several Cupressus plants[31].

C-C Linked Biflavonoids:

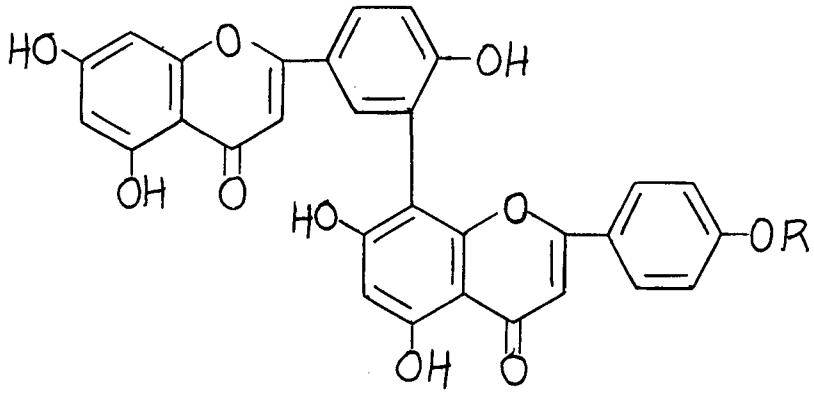
Cupressuflavone (LXIII) has been isolated from C.funebris[33], C.goveniana[33, 34] C.sempervirens[33, 37] C.torulosa[33, 37] C.australis[35] C.lusitanica[36] and 4'-O-methylcupressuflavone (LXIV) has been reported from C.goveniana[34] and C.lusitanica[36].



(LXIII) Cupressuflavone R = H

(LXIV) 4'-O-methyl-cupressuflavone R = CH_3

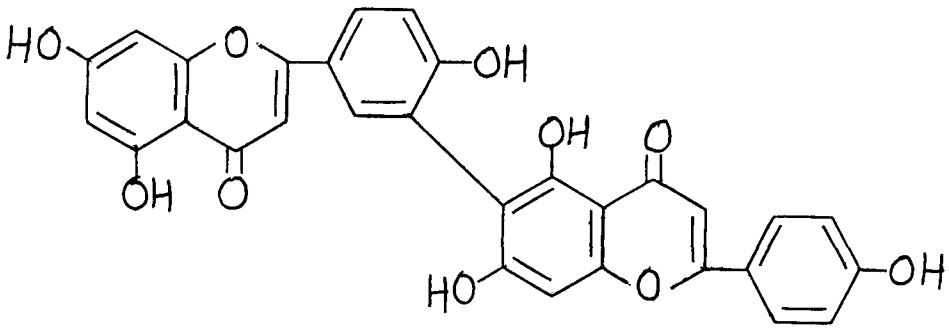
Amentoflavone (LXV) has been isolated from *C.funebris*[33], *C.coveniana*[33,34], *C.sempervirens*[33] *C.australis*[35] and *C.lusitanica*[36] and mono-O-methylamentoflavone has been isolated from *C.australis*[35]. Podocarpusflavone (LXVI) occurs in *C.lusitanica*[36].



(LXV) Amentoflavone R = H

(LXVI) Podocarpusflavone R = Me

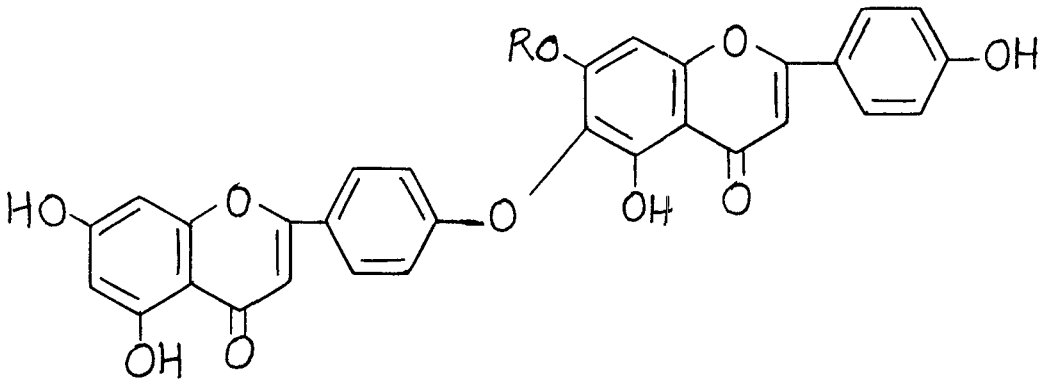
Robustaflavone (LXVII) has been reported from *C.australis*[35].



(LXVII)

C-O-C Linked Biflavonoids:

Hinokiflavone (LXVIII) has been isolated from C.arizonica[37], C.funebris[33], C.torulosa[33], C.sempervirens[33], C.goweniana[33,34] and mono-O-methyl hinokiflavone detected in C.australis[35], C.lusitanica[36]. Isocryptomerin (LXIX) has been reported from C.funerbis[33], C.goweniana[33,34] C.sempervirens[33].



(LXVIII) Hinokiflavone R = H

(LXIX) Isocryptomerin R = CH_3

DISCUSSION

Biflavonoids from Cupressus funebris

A number of Cupressus species have been examined and amentoflavone and cupressuflavone were reported as the characteristic biflavone of the genus, which may serve as the useful taxonomic marker[31-37]. Amentoflavone, Cupressuflavone and hinokiflavone have been reported from the leaves of Cupressus funebris. Leaves extracts of Cupressus funebris were reinvestigated. Mono-o-methyl amentoflavone, mono-o-methyl robustaflavone, mono-o-methyl hinokiflavone and di-o-methyl robustaflavone have been isolated and identified in addition to three biflavone reported earlier[32].

Isolation and Purification

The phenolic extractive from the fresh dried leaves of Cupressus funebris procured from the Kathmandu valley (Nepal) were purified by solvent fractionation and column chromatography on silica gel (Petrol, benzene, Et_2O, EtOAc (9:1, 8:2, 7:3, 1:1). Yellow solid mass responded to the flavonoidic colour test (HCl/Zn). The 2DC (BAF 36:9:5) examination of the purified product revealed the presence of five bands which were labelled as CF_I (R_f 0.14), CF_{II} (R_f 0.25), CF_{III} (R_f 0.34), CF_{IV} (R_f 0.44) and CF_V (R_f 0.55). The bands CF_I, CF_{III} and CF_V correspond to amentoflavone and its mono- and dimethylethers.

While CF_{II} and CF_{IV} are comparable to mono-and dimethyl ethers of Robustaflavone, respectively (Fig.I). Each band was separated by PTLC on Silica gel (Benzene / Pyridine formic acid, 36:9:5) after purification by column chromatography. The R_f values and characteristic shades in UV light of parent biflavones and their methyl ethers provide a method of fairly accurate identification.

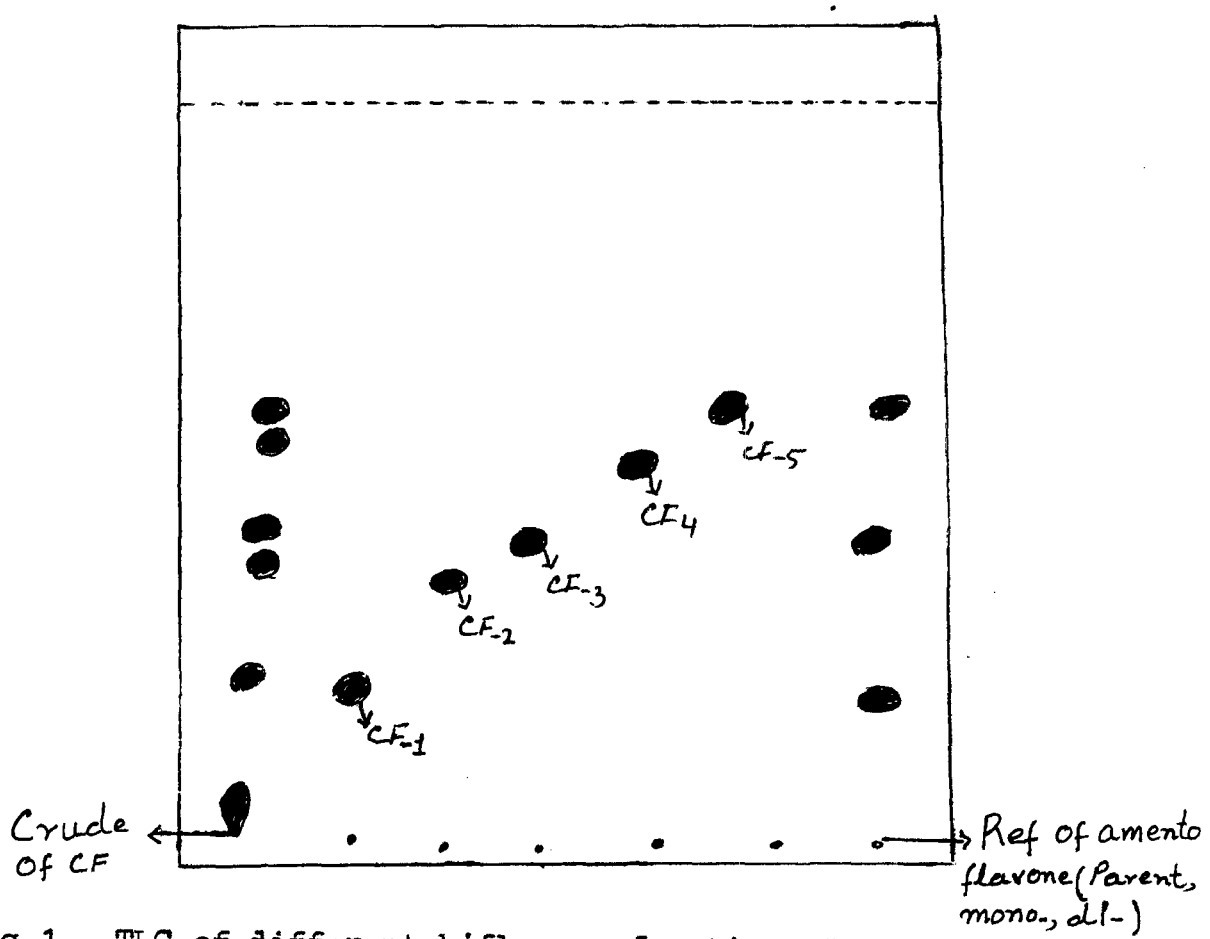


Fig.1. TLC of different biflavone fractions C-funebris (Si-gel) was used as adsorbent and BPF as developer

CF_I :-

The band CF_I, on methylation (by dimethylsulfate and dried acetone) and TLC examination was found to be

the mixture of two methyl ethers, amentoflavone hexamethyl ether and cupressuflavone hexamethyl ether which were identified by comparasion with authentic samples and characteristic shales in UV light.

CF_2 :-

On methylation and TLC examination, CF_2 showed the presence of monoethyl ether of Robustaflavone by comparison with authentic sample and characteristic florescence in UV light.

CF_3 :-

The fraction CF_3 on methylation followed by TLC examination revealed the presence of amentoflavone hexamethyl ether and hinokiflavone pentamethyl ether, on comparasion with authentic samples (R_f values and characteristic shales in UV light). Therefore CF_3 was identified as the mixture of mono-o-methyl amentoflavone and hinokiflavone.

CF_4 :-

The methylated prolust of the fraction CF_4 was found to be similar to robusta flavone hexamethyl ether on comparasion with authentic sample (R_f value and characteristic flouresscence in UV light). CF_4 was therefore identified as di-o-methyl robustaflavone.

CF_5 :-

The fraction CF_5 on methylation followed by TLC examination was found to be penta-O-methyl hinokiflavone, identified by the comparasion with the authentic sample. CF_5 is, therefore, mono-O-methyl hinokiflavone.

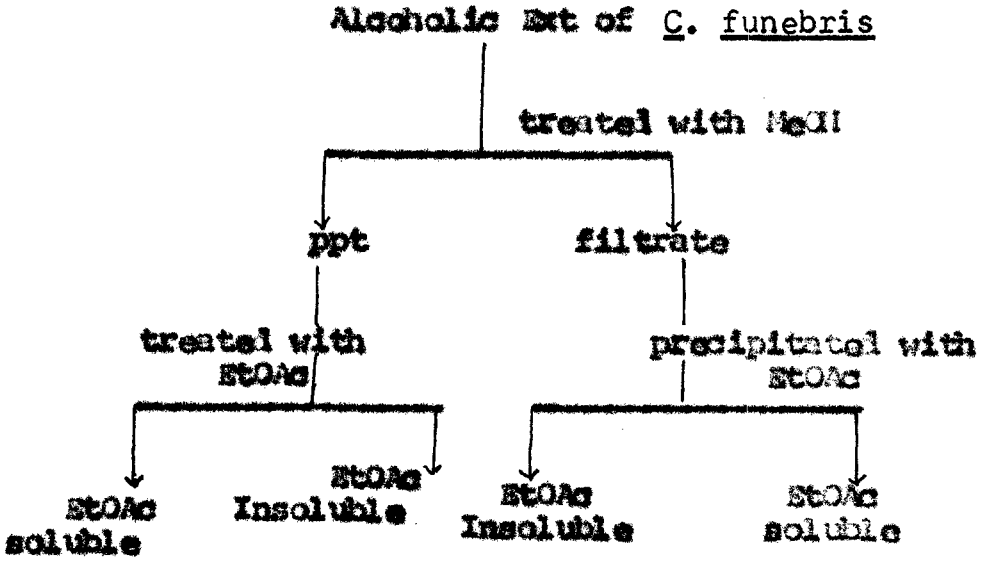
Further characterization of the isomeric mixtures is in progress.

EXPERIMENTAL

Isolation and Characterisation of Biflavonoils from Cupressus funebris

Extraction:

The dried leaves were exhaustively treated with EtOH. The EtOH extract was dried under reduced pressure. Alcoholic extract was treated with methanol, the two fractions were obtained as shown below.



The EtOAc soluble fraction giving usual flavonoidic colour test, were concentrated first at atmospheric pressure and then under reduced pressure, giving the gummy greenish mass. This was refluxed with petroleum ether (40-60°), benzene successively till the solvent in each case was almost colourless. The petrol extract and benzene extract showed no flavonoidic colour reaction

and were rejected. The residue was then refluxed with EtOAc which responded to the usual colour reaction for flavonoids.

Purification of Biflavonoid Mixture-Column Chromatography

A well stirred suspension of silica-gel (200 gm) in dry petroleum ether (40-60°) was poured into a column (150 cm long and 50 mm in diameter). When the adsorbent was well settled, the petroleum ether was allowed to pass through the column. The crude mixture of biflavonoid adsorbed on silica gel was added to the column. The column was successively eluted with petroleum ether, benzene, benzene-ethylacetate (9:1, 8:2, 7:3, 6:4, 1:1), ethyl acetate, acetone. Benzene-ethylacetate fractions (9:1, 8:2, 7:3, 6:4, 1:1) gave yellow crude biflavonoids. The TLC (BPF 36:9:5) examination of the purified product revealed the presence of five bands which were labelled as CF_1 (R_f 0.14), CF_2 (R_f 0.25), CF_3 (R_f 0.34), CF_4 (R_f 0.44) and CF_5 (R_f 0.55).

Separation of Biflavonoid mixture-Preparative thin layer chromatography

The glass plate (20x20 cm) were coated with a well stirred suspension of silica gel (35 gm, 70 ml, water)

in order to obtain thin layer on plate. After drying the plates for 2 hrs at room temperature, the plates were activated at 110-120° for 1 hr. The flavonoidic fraction was then subjected to preparative thin layer chromatography using BPF (36:9:5) as the developing solvent. The position of the bands were marked in U.V. light. The marked pigment zones were scraped with the help of spatula and eluted in separate columns with dry acetone. The solvent was recovered till the eluent was reduced to 20-30 ml. The addition of water and HCl yielded the yellow precipitate in each case. The precipitate was filtered, washed with water and dried. Homogeniety of the bands was checked by TLC. The components were labelled as CF_1, CF_2, CF_3, CF_4, CF_5. Each component was checked by methylation for accurate identification.

Characterisation (Preparation of derivative)

Methylation of CF_1:-

CF_1 (50 mg), anhydrous potassium carbonate (1 gm), dimethyl sulfate (0.5 ml) and dried acetone (100 ml) were refluxed on water bath for 6 hrs. A small portion of the reaction mixture was taken out and tested for alcoholic $FeCl_3$ reaction. Refluxing continued until it gave a

negative alcoholic $FeCl_3$ test. It was then filtered and evaporated to dryness. The yellow oily mass left behind was treated with petroleum ether and then dissolved in chloroform. The chloroform solution was washed with water, dried over anhydrous Na_2SO_4 and concentrated to give a crude solid. The methylated mixture by TLC examination showed the presence of two methyl ethers, amentoflavone hexamethylether and cupressuflavone hexamethylether which were identified by comparasion with with authentic (R_f value and characteristic shades in U.V.light).

CF_2permethylated derivatives:-

On methylation CF_2 gave robustaflavone hexamethyl-ether, identified by comparison with authentic sample.

CF_3 permethylated derivatives:-

The fraction CF_3, on methylation, followed by TLC examination, revealed the presence of amentoflavone hexamethylether and hinokiflavone pentamethylether, on comparison with authentic samples (R_f values and characteristic shades in U.V. light).

CF_4 permethylated derivatives:-

The methylation of fraction CF_4 was done as in

the case of CF_1. The robusta flavone hexamethylether was obtained as product, which was compared with authentic sample (R_f value and characteristic fluorescence in U.V.light).

CF_5 permethylated derivatives:-

The fraction CF_5 was methylated as in the case of CF_1. The hinokiflavone pentamethylether was the only product which was identified by the comparison with authentic sample.

PART - B

INTRODUCTION

Araucaria belongs to the family Araucariaceae and comprises two genera of about 32 species.

Chemical Constituents of *Araucaria*

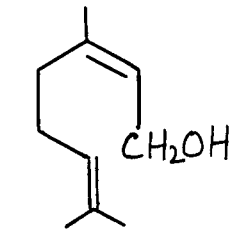
Most of the *Araucaria* plants investigated so far constitute biflavonoids and diterpenoids. Some *Araucaria* (referred as A. in the subsequent paragraph) plants also contain lignans, resinols, monoterpenes and sesquiterpenes as well.

Terpenoids

Terpenoids are the most frequently isolated natural products in the essential oil and oleoresin of *Araucaria* plants. However, diterpenes are more common than monoterpenes and sesquiterpenes.

Monoterpenes

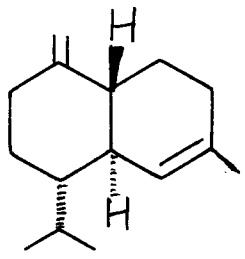
Monoterpene with p-menthane skeleton, limonene (XXI), occurs in *A. araucana* essential oil[39] and *A. bidwillii* exudate[40]. Geraniolene (LXX) has also been reported from *A. araucana*[39]. Thujane and Pinane skeleton, common in *cupressus* and *pinus*, were absent in *Araucaria*.



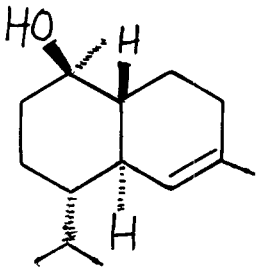
(LXX)

Sesquiterpenes

Cadinanes, (+)-γ-Cadinene (LXXI) and (-)-α-Cadinol (LXXII) have been reported from the bark of A. araucana[39].



(LXXI)



(LXXII)

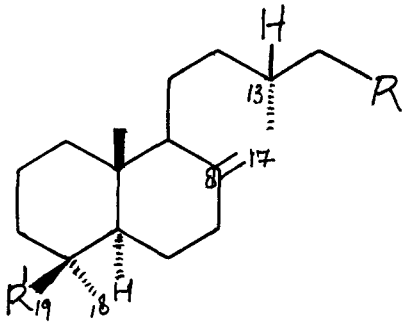
Diterpenes

Diterpenes with labdane skeleton are more frequent in Araucaria than those with other skeleton. Almost all the Araucaria plants examined so far contain bicyclic labdane

derivatives. Tricyclic and tetracyclic diterpenes have also been isolated from some species.

Bicyclic diterpenes

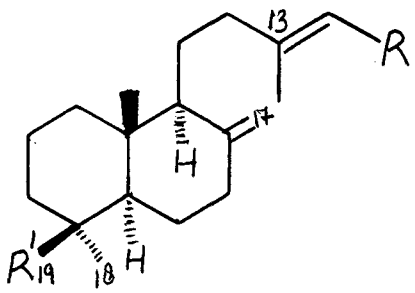
Labdanes



		R	R'
(LII)	Imbricatolic acid	CH_2OH	CO_2H
(LIII)	15-Acetoxyimbricatolic acid	CH_2OAc	CO_2H
(LXXIII)	Imbricatadiol	CH_2OH	CH_2OH
(LXXIV)	Imbricatolal	CH_2OH	CHO
(LXXV)	15-Acetoxyimbricatalolal	CH_2OAc	CHO
(LXXVI)	15-Acetoxylab-8(17)-ene	CH_2OAc	CH_3
(LXXVII)	15-Hydroxylabda-8(17)-ene-19-oate	$-CHOH$	CH_2OAc
(LXXVIII)	Me-15-acetoxylab-8(17)-ene-19-oate	$-CH(CH_3)-OAc$	COOMe
(LXXIX)	15-Hydroxylabd-8(17)-ene	CH_2OH	CH_3

Imbricatolic acid (LII), a constituent from A. angustifolia[41], occurs along with acetyl acid (LIII), diol (LXXIII), hydroxyaldehyde (LXXIV) and acetoxyaldehyde (LXXV) in A. araucana[42] which is the only other plant to contain labdanes with saturated side chain at C_9.

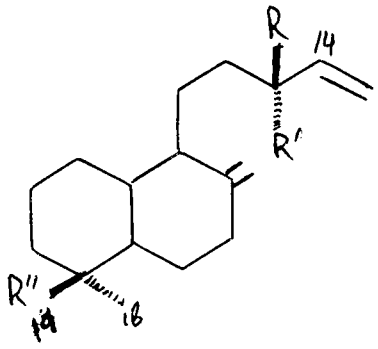
Diterpenes with 8(17),13-labdadiene skeleton, such as agathic acid (LXXX) was found to occur in A. angustifolia[41], while its dimethyl ester (LXXXI) occurs in A. bidwillii[43]. Agatholic acid (LXXXII) was present only in A. angustifolia[41]. A. bidwillii[43] constitutes hydroxy ester (LXXXIII) and acetoxy ester-acetyl agatholate (LXXXIV). Agathadiol (LXXXV) occurs along with acetyl-isocupressic acid (LXXXVI) and methyl-isocupressate (LXXXVII) in the oleoresin of A. cookii[44]. In A. cunninghamii[44] methylacetylisocupressate (LXXXVIII) was isolated together with methylisocupressate (LXXXVII). Aldehydo ester methyl agathalate (LXXXIX) from A. bidwillii[43] is the only example of occurrence of aldehyde with 8(17),13-labdadiene skeleton in Araucaria .



		R	R'
(LXXX)	Agathic acid	CO_2H	CO_2H
(LXXXI)	Dimethylagathate	CO_2Me	CO_2Me
(LXXXII)	Agatholic acid	CO_2H	CH_2OH
(LXXXIII)	Methyl agatholate	CO_2Me	CH_2OH
(LXXXIV)	Acetyl agatholate	CO_2Me	CH_2OAc
(LXXXV)	Agatha diol	CH_2OH	CH_2OH
(LXXXVI)	Acetyl-isocupressic acid	CH_2OAc	CO_2H
(LXXXVII)	Methylisocupressate	CH_2OH	CO_2Me
(LXXXVIII)	Methyl-acetylisocupressate	CH_2OAc	CO_2Me
(LXXXIX)	Methyl agathalate	CO_2Me	CHO

Diterpenes with 13-hydroxy-8(17),14-labdadiene skeleton, cupressic acid (XLVII) and its methyl ester (XC) were found in A. excelsa[45] and A. cookii[44], respectively. Acetoxy ester (XCI) was found only in A. cookii[44].

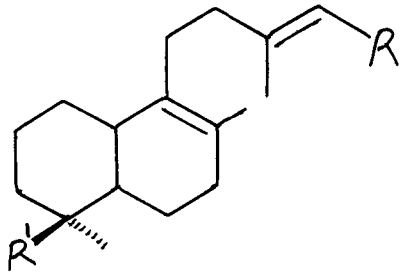
Torulosol (XLIV) was isolated from A. excelsa oleroresin[45], while its isomer epitorulosol (XCIII) is a constituent of A. cookii[44]. Torulosal (XLV), a constituent of A. cookii[44], occurs along with hydrocarbon, manool (XLIII) in A. excelsa[45]. Compound XCII has been reported from A. araucana[46].



		R	R'	R''
(XLVII)	Cupressic acid	OH	CH_3	CO_2H
(XC)	Methyl-13,R-hydroxy-8(17),14-labdadiene-19-oate	OH	CH_3	CO_2Me
(XCI)	13R-acetoxy-8(17),14-labda-19-oic acid	OAc	CH_3	CO_2H
(XCII)	13R-acetoxy-8(17),14-labdadiene-19-oate	OAc	CH_3	CO_2Me
(XLIV)	Torulosol	CH_3	OH	CH_2OH
(XCIII)	Epitorulosol (8(17),14-labdadiene-13R,19-diol)	OH	CH_3	CH_2OH
(XLV)	Torulosal (13R-hydroxy-8(17),14-labdadiene-19-al)	OH	CH_3	CHO
(XLIII)	Manool	OH	CH_3	CH_3

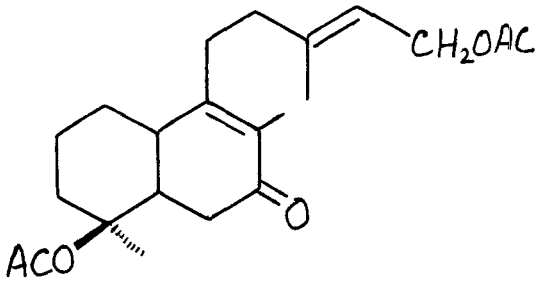
8,E-13-labdadienes are found as hydroxy acid (XCIV), acetoxy ester (XCV), diol (XCVII) and its mono-acetate (XCVIII) and diacetate (XCIX), hydroxy aldehyde (C) and

acetoxyaldehyde (CI) in the oleoresin of A. cunninghamii[44]. Acetoxy ester (XCVI) and hydroxyaldehyde (C) was also found in A. cookii[44]. This skeleton is surprisingly absent in other Araucaria spp.



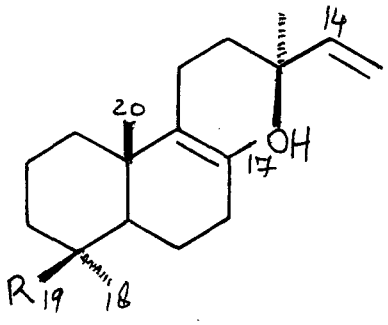
		R	R'
(XCIV)	15-Hydroxy-8,E-13-labdadiene-19-oic acid	CH_2OH	CO_2H
(XCV)	Methyl-15-acetoxy-8,E-13-labdadiene-19 oate	CH_2OAc	CO_2Me
(XCVI)	Methyl-19-hydroxy-8,E-13-labdadiene-15-oate	CO_2Me	CH_2OH
(XCVII)	8,E-13-labdadiene-15,19-diol	CH_2OH	CH_2OH
(XCVIII)	15-acetoxy-8,E-labdadiene-19-ol	CH_2OAc	CH_2OH
(XCIX)	8,E-13-labdadiene-15,19-diacetate	CH_2OAc	CH_2OAc
(C)	15-Hydroxy-8,E-labdadiene-19-al	CH_2OH	CHO
(CI)	15-Acetoxy-8,E-labdadiene-19-al	CH_2OAc	CHO

Caputo et al.[44] found 7-oxo-acetoxy ester (CII) in A. cunninghamii, possibly an artefact formed via allylic oxidation of acetoxy ester (XCV).



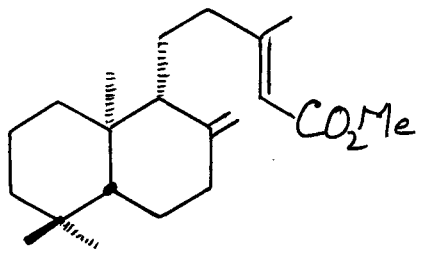
(CII) Methyl-7-oxo-19-acetoxy-8,E-13-labdadiene-15-oate

Hydroxy ester (CIII), hydroxyaldehyde (CIV) and diol (CV) were found to be present in A. cookii[44].

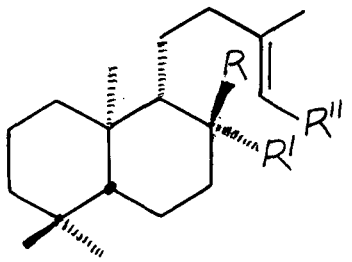


		R
(CIII)	Methyl-13(R)-hydroxy-8,14-labdadiene-19-oate	CO_2Me
(CIV)	13(R)-hydroxy-8,14-labdadiene-19-al	CHO
(CV)	8,14-labdadiene-13(R),19-diol	CH_2OH

So far, we have dealt with the labdanes having the normal absolute configuration. In two interesting reports, Caputo and Mangonii (1974)[43] and Caputo et al. (1976)[46] have observed that the labdanes carrying oxygenated function at C_{19} have the normal configuration, whereas those having C_{19} methyl group posses the enantiemeric configuration. ent-Labdane ester (CVI), hydroxy ester (CVII) and (CVIII), diol (CIX), alcohol (CX) and its acetate (CXI) were all isolated from acidic and neutral fractions of only A. bidwillii resin[43,46]. Another unusual alcohol with nor-labdane skeleton,



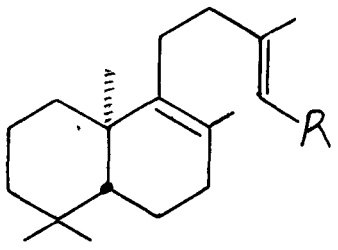
(CVI)



(CVII) Methyl-ent-8α-hydroxylabda-E-13-ene-15-oate.
(R = OH, R' = CH_3, R'' = CO_2Me)

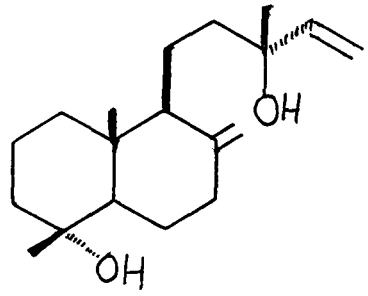
(CVIII) Methyl-ent-8β-hydroxylabda-E-13-ene-15-oate.
(R = CH_3, R' = OH, R'' = CO_2Me)

(CIX) ent-8β,15-labda-E-13-ene diol.
(R = CH_3, R' = OH, R'' = CH_2OH)

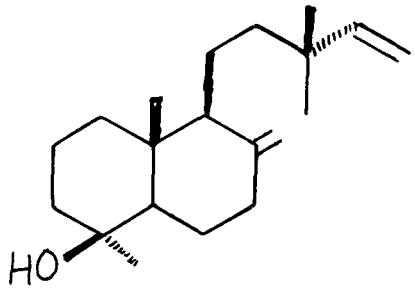


		R
(CX)	ent-labda-8,E-13-diene-15-ol	CH_2OH
(CXI)	ent-15-acetoxylabda-8,E-13-diene	CH_2OAc

18-nor-labda-8(17),14-diene-4α,13(R)-diol (CXII) occurs with its 4β-hydroxyisomer (CXIII) in the oleoresin of A. excelsa[45]. It is interesting to note that no other plant of Araucariaceae was found to contain nor-labdanes.



(CXII)

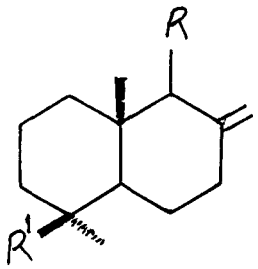


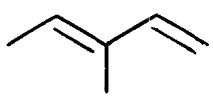
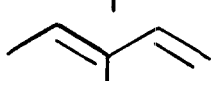
(CXIII)

(CXII) 18-nor-labda-8(17),14-diene-4α,13-diol.

(CXIII) 19-nor-labda-8(17),14-diene-4β,13-diol.

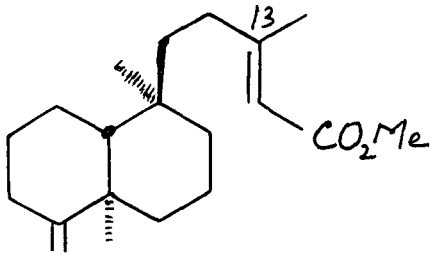
Labdatrienes, trans-communic acid (L) was found in A. excelsa[45] and its ester (LI) in A. cunninghamii[44].



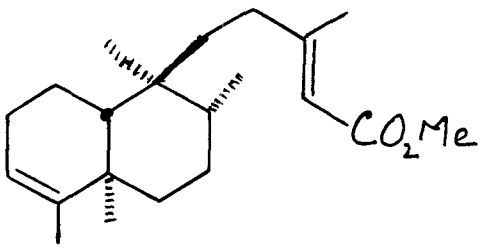
		R	R'
(L)	trans-communic acid		CO_2H
(LI)	Methyl-trans-communate		CO_2Me

Clerodanes

Two diterpene ester ent-4(18),E-13-cleroda-diene-15-oate (CXIV) and ent-3,E-13-cleroda-diene-15-oate (CXV) were isolated from oleoresin of *A. bidwillii*[43] and this represents the first case of co-occurrence of clerodanes with labdane skeleton, a fact of immense biogenetic significance.



(CXIV)

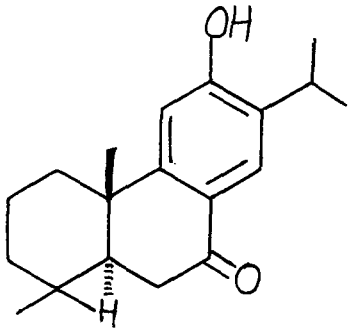


(CXV)

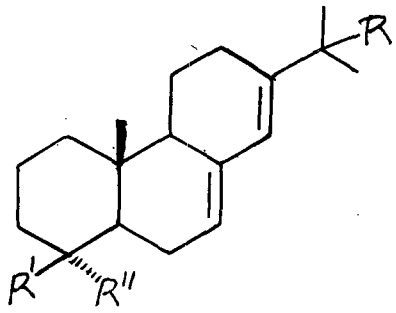
Tricyclic diterpenes

Abietanes and Pimaranes

Sugiol (CXVI) from the bark of *A. angustifolia*[41], abietinol (CXVII) and abietic acid (CXVIII) from the oleoresin of *A. cookii*[44] and *A. excelsa*[45] are the only examples of occurrence of this series in *Araucaria*.



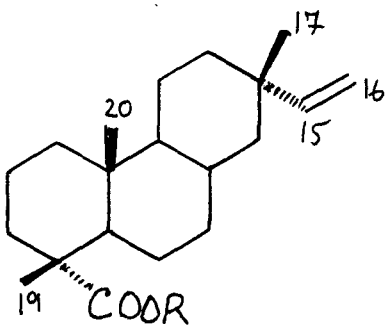
(CXVI)



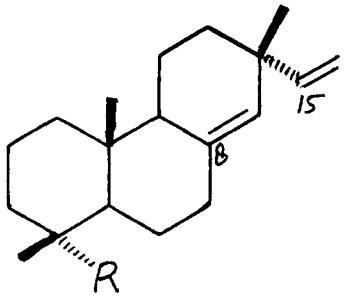
(CXVIII)
(R=H, R'=CH$_3$, R''=COOH)

(CXVII)
(R=H, R'=CH$_3$, R''=CHO)

Sandaracopimaric acid (LIX) and sandaracopimaradienol (CXIX) were also found to occur in A. excelsa and A. cookii, respectively.



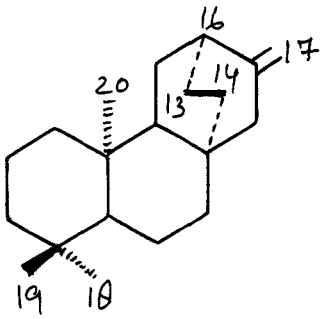
	R
(LIX) Sandaracopimeric acid	H



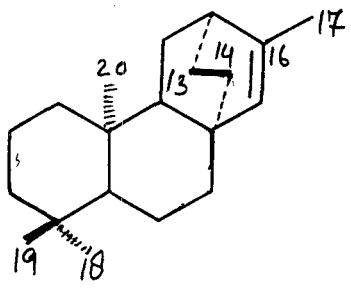
		R
(CXIX)	Sandaracopimaradienol	CH_2OH

Tetracyclic diterpenes

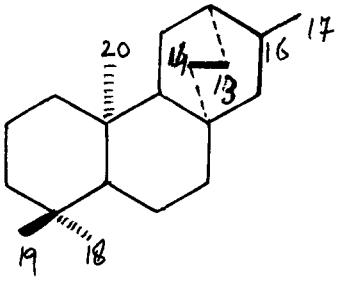
In an interesting paper, Briggs and White have reported the occurrence of (-)-atisirene (CXX), isoatisirene (CXXI), (-)-tetracyclobane (CXXII) and (+)-hibaene (LXI) from the bark of A. araucana[39]. Further investigations on the bark of other Araucariaceae plants seems promising.



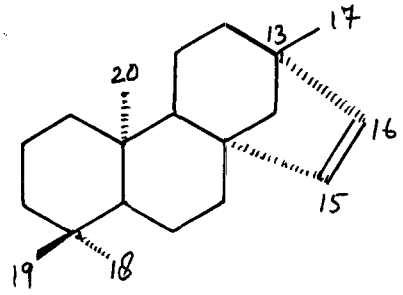
(CXX)



(CXXI)

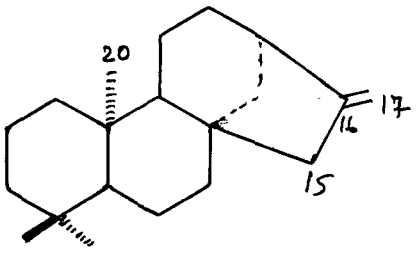


(CXXII)

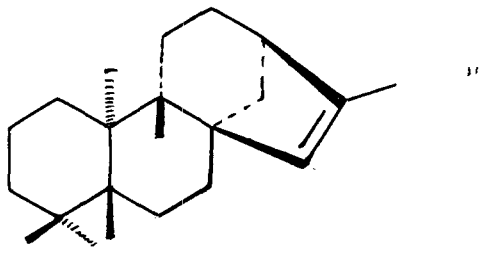


(LXI)

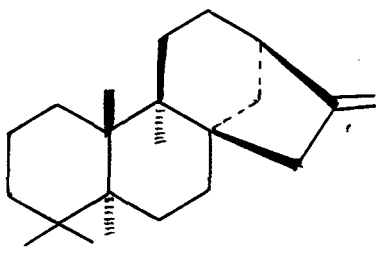
The bark of A. araucana[39] contains (-)-kaurene (CXXIII) and isokaurene (CXXIV). A typical constituent of conifers, phyllocladene (LXII) was reported only in A. rulei, F. Mull[47]. In Araucariales, absence of d-kaurene, a constituent of podocarpus ferrugineus is noteworthy.



(CXXIII)



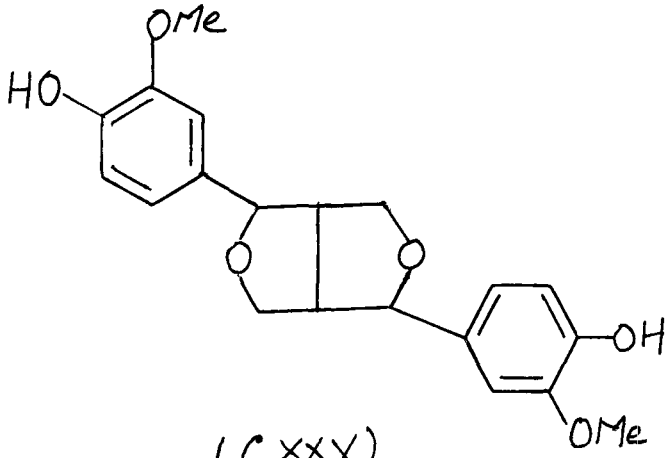
(CXXIV)



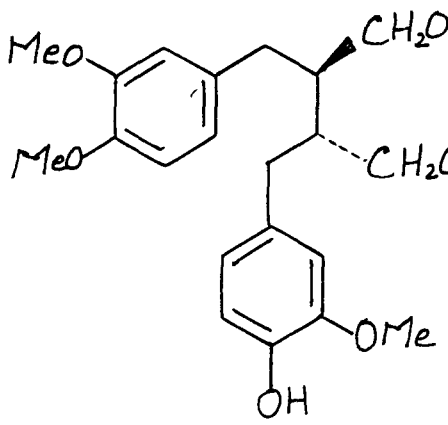
(LXII)

Lignins

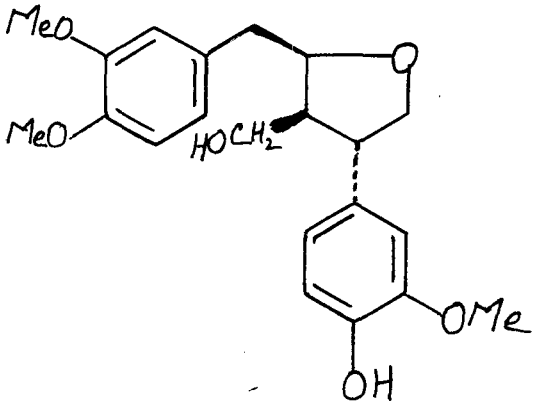
An interesting group of phenols containing seventeen carbon atoms, pinoresinol (CXXV)[48], isolariciresinol[48], seco-isolariciresinol[48], lariciresinol[48], seco-isolariciresinol monomethyl ether (CXXVI)[49] and lariciresinol-4-methyl ether (CXXVII)[49] have been isolated from Araucaria angustifolia.



(CXXV)



(CXXVI)

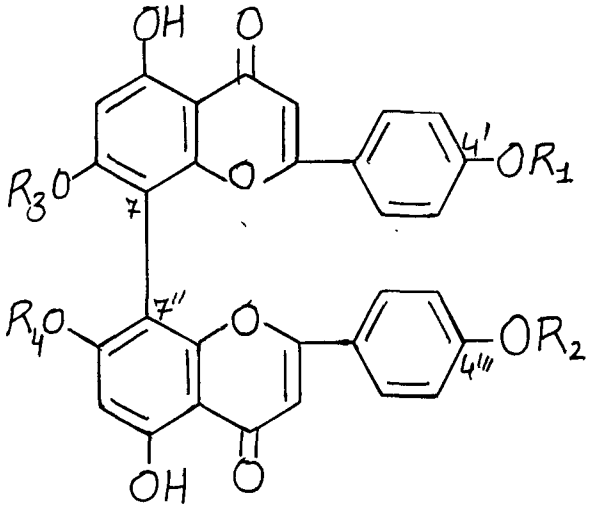


(CXXVII)

Biflavonoids

Several Araucaria plants have been investigated after the first report of biflavone, WB_1 (CXXXII) from the leaves of A. cunninghamii[50] by Rahman and Bhatnager in 1968.

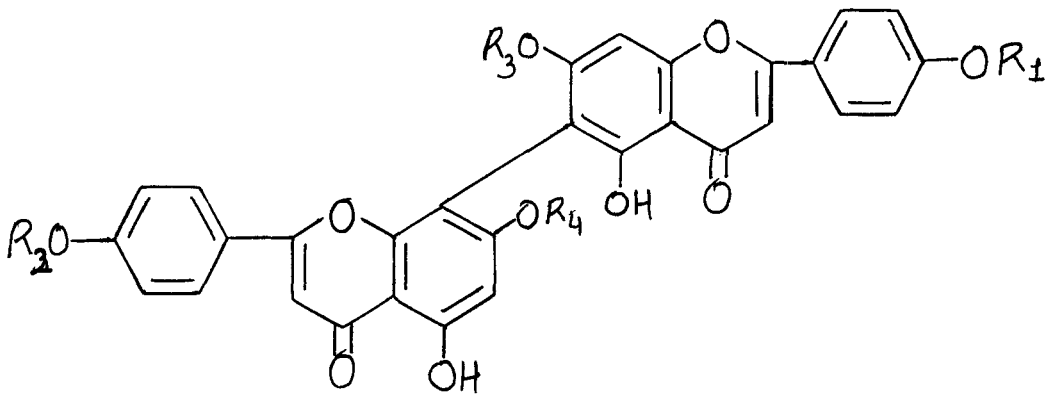
7-O-methylcupressuflavone (CXXVIII) and 7,7"-di-O-methylcupressuflavone (CXXIX) occur in the leaves of A. bidwillii[51,52] and A. rulei[53]. A. cunninghamii[54] and A. cookii[55] constitute dimethylethers CXXIX and CXXX along with trimethylether, CXXXI and tetramethylether, CXXXII.



		R_1	R_2	R_3	R_4
(LXIII)	Cupressuflavone	H	H	H	H
(CXXVIII)	7-O-Methyl-	H	H	CH_3	H
(CXXIX)	4'/4"',7-Di-O-methyl-	CH_3	H	CH_3	H
	or	H	CH_3	CH_3	H

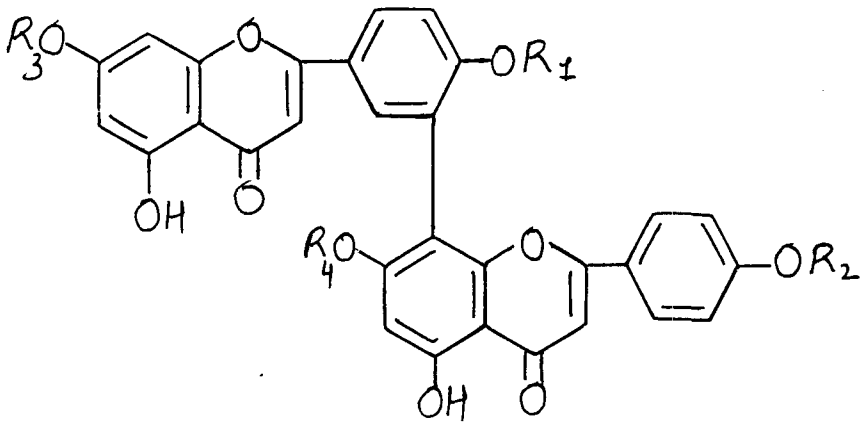
		R_1	R_2	R_3	R_4
(CXXX)	4'/4"',7-Di-O-methyl-ether	CH_3	H	CH_3	H
		H	CH_3	CH_3	H
(CXXXI)	4',7,7"-Tri-O-methyl-	CH_3	H	CH_3	CH_3
(CXXXII)	4',4"',7,7"-Tetra-O-methyl-	CH_3	CH_3	CH_3	CH_3

7-O-methylagathisflavone (CXXXIV) and 7,7"-di-O-methylagathisflavone (CXXXV) were reported from *A. bidwillii*[51,52] while in *A. cunninghamii*[54,55], monomethylether, (CXXXIV) exists along with the 4"',7-di-O-methylagathisflavone (CXXXVI). Dimethylethers, CXXXV and CXXXVI were also isolated from *A. angelea*[53] and *A. vitiensi*[53], respectively. Tri-O-methylagathisflavone (CXXXVII) could be identified only in *A. angelea*[56] although it has been detected along with agathisflavone (CXXXIII) in other species as well[57].



		R_1	R_2	R_3	R_4
(CXXXIII)	Agathisflavone	H	H	H	H
(CXXXIV)	7-O-Methyl-	H	H	CH_3	H
(CXXXV)	7,7"-Di-O-methyl-	H	H	CH_3	CH_3
(CXXXVI)	4"',7-Di-O-methyl-	H	CH_3	CH_3	H
(CXXXVII)	4"',7,7"-Tri-O-methyl-	H	CH_3	CH_3	CH_3

Amentoflavone monomethylether, bilobetin (CXXXVIII) is the sole constituent of this series in A. bidwillii[51,52]. A. seekii[55] constitutes all the four derivatives, monomethylether (CXXXIX), dimethylether (CXL), trimethylethers (CXLII and CXLIII) and tetramethylether (CXLV). A. excelsa[56] constitutes another set of mono-, di-, trimethylethers(CXXXIX, CXLI and CXLIV, respectively and tetramethylether. A. cunninghamii[54] contains di-, tri- and tetramethylethers CXL, CXLIII and CXLV, respectively.



		R_1	R_2	R_3	R_4
(LXV)	Amentoflavone	H	H	H	H
(CXXXVIII)	Bilobetin	CH_3	H	H	H
(CXXXIX)	7"-O-Methyl-	H	H	H	CH_3
(CXL)	4',7"-Di-O-methyl-	CH_3	H	H	CH_3
(CXLI)	7,7"-Di-O-methyl-	H	H	CH_3	CH_3
(CXLII)	Kayaflavone	CH_3	CH_3	H	CH_3
(CXLIII)	Sciadopitysin	CH_3	CH_3	CH_3	H
(CXLIV)	4',7,7"-Tri-O-methyl-	CH_3	H	CH_3	CH_3
(CXLV)	4',4"',7,7"-Tetra-O-methyl	CH_3	CH_3	CH_3	CH_3

Biphenylether type of biflavone, himekiflavone (LXVIII) has also been identified in *A. sieboldii*[57].

DISCUSSION

Biflavonoids from the leaves of *Araucaria cookii* R.Br. Ex. D. Don

The leaves of *A. cookii* were procured from Vice-Chancellor's Lodge, Aligarh Muslim University, Aligarh, India. Extraction of the fresh leaves followed by solvent fractionation and preparative TLC gave nine fractions labelled as Ac_1-Ac_9. The usual colour reaction and other diagnostic test indicated all of them to be flavonoids. The complexities of different fractions were studied by thin layer chromatographic examination of their methylated products.

The isolation and characterization of 7,7"-di-O-methylcupressuflavone (CXXIX), kayaflavone (CXLII), 4',4"',7,7"-tetra-O-methylamentoflavone (CXLV) and 4',4"',7,7"-tetra-O-methylcupressuflavone (CXXXII) as major constituents of leaves extract of *A. cookii* have been reported by Pelter et al.[55] The isolation and characterization of some minor constituents, such as, hinokiflavone (LXVIII) and 7"-O-methylamentoflavone (CXXXIX), 4"',7-di-O-methylagathisflavone (CXXXVI), 4',7"-di-O-methylamentoflavone (CXL), sciadopitysin (CXLIII) and 4',7,7"-tri-O-methylcupressuflavone (CXXXI) were also reported[58]. The present discussion deals with the systematic identification of some minor biflavones in addition to ten biflavones reported earlier[55,58]. The reinvestigation of the leaf extracts of *A. cookii* revealed the presence of agathisflavone (CXXXIII), cupressuflavone (LXIII), amento-

flavone (LXV), robustaflavone (LXVII), agathisflavone monomethylether and robustaflavone monomethylether which were isolated and identified on TLC.

The TLC examination of purified product revealed the presence of nine fractions which were labelled as Ac_1 (R_f, 0.16) Ac_2 (R_f, 0.17), Ac_3 (R_f, 0.26), Ac_4 (R_f, 0.35), Ac_5 (R_f, 0.44), Ac_6 (R_f, 0.51), Ac_7 (R_f, 0.70), Ac_8 (R_f, 0.77) and Ac_9(R_f,0.78).

The bands Ac_1, Ac_3, Ac_5 correspond to the agathisflavone and its mono- and dimethylethers and Ac_2, Ac_4, Ac_6 and Ac_7 bands correspond to the amentoflavone, its mono-, di- and trimethylethers, the band Ac_8, Ac_9 correspond to the tetramethylethers of amentoflavone and cupressuflavone, respectively, as shown in Fig. 2. Each band was separated and purified by column chromatography followed by preparative-layer-chromatography using Benzene:Pyridine:Formic acid (36:9:5) as the developing solvent system.

Ac_1

Thisfraction has same R_f values and shades as that of parent agathisflavone and robustaflavone. It was methylated with dimethylsulfate in driedacetone. TLC examination of methylated product revealed the presence of agathisflavone hexamethylether and robustaflavone hexamethylether (R_f values and characteristic shades in U.V. light). Therefore, Ac_1 was identified as the mixture of parent agathisflavone and robustaflavone.

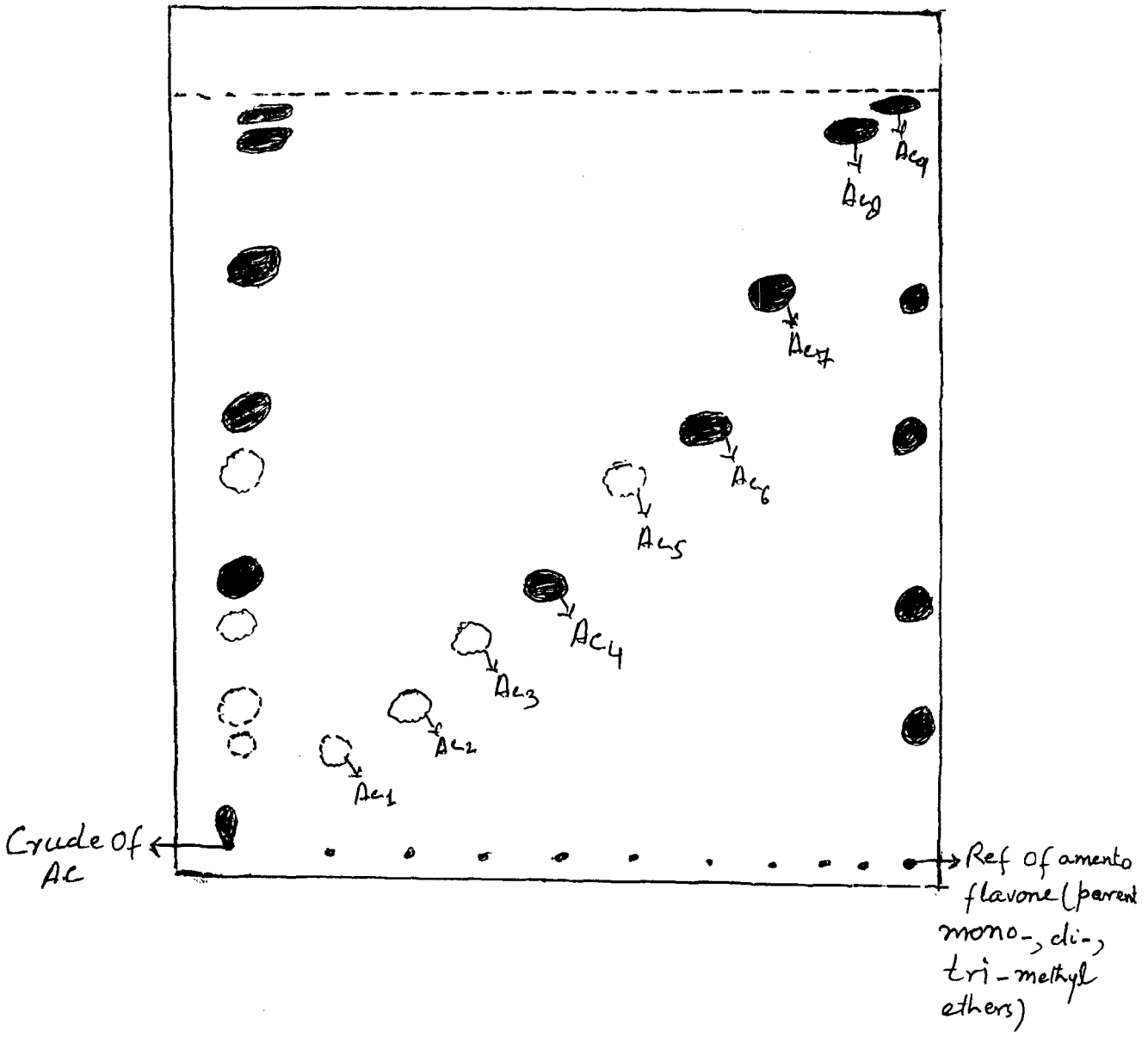


Fig. 2: TLC of different biflavone fractions of *A. cookii* (Silica gel was used as adsorbent and BPF as developer).

Ac_2

This fraction resembles parent amentoflavone and cupressuflavone on TLC. On methylation and TLC examination of the methylethers, amentoflavone hexamethylether and cupressuflavone hexamethylether were detected (comparison of R_f values of authentic samples and characteristic shades in U.V. light). Therefore, Ac_1, is an isomeric mixture of amentoflavone and cupressuflavone.

Ac_3

It resembles with the monomethylether of agathisflavone on TLC (R_f values and shades). On methylation it gave agathisflavone hexamethylether and robustaflavone hexamethylether which were identified by comparison with the authentic samples (R_f values and shades in U.V. light). Ac_3 is, therefore, a mixture of agathisflavone monomethylether and robustaflavone monomethylether.

Ac_4, Ac_5, Ac_6, Ac_7, Ac_8 and Ac_9

Fractions, Ac_3-Ac_9 were compared with the authentic samples isolated earlier[58]. Their permethylated derivatives were also prepared by reaction with dimethylsulfate in acetone and the methylethers were compared with the authentic

permethylated C-C linked biflavones. The results were in conformity with the earlier characterization.

The isomeric mixtures identified in fractions Ac_1, Ac_2 and Ac_3 are under investigation by HPLC.

EXPERIMENTAL

Isolation and Characterization of Biflavonoids from *Araucaria cookii*

Extraction

The dried leaves of *Araucaria cookii* were extracted with EtOH, the EtOH extracts was dried first at atmospheric pressure, then under reduced pressure. The dried mass was refluxed with petroleum ether (40-60°), benzene, chloroform, ethylacetate, acetone successively till the solvent in each case was almost colourless. The chloroform and EtOAc extracts were mixed together which on concentration gave the dark brown solid which responded to the usual flavonoidic colour test.

Purification of Biflavonoid Mixture-Column Chromatography

A well stirred suspension of Si-gel (150 gm) in dry petroleum ether (40-60°) was poured into a column (150 cm long and 50 mm in diameter). When the adsorbent was well settled the excess of petroleum ether was allowed to pass through the column. The crude mixture of biflavonyl adsorbed on Si-gel was added to the column. The column was eluted with the organic solvents in order of increasing polarity, petroleum ether, benzene, benzene:ethyl acetate (9:1, 8:2, 7:3, 6:4, 1:1), ethyl acetate, acetone, methanol. The flavonoidic fractions eluted by C_6H_6:EtOAc (9:1, 8:2, 7:3, 6:4, 1:1) gave

nine spots on TLC (BPF 36:9:5). They were labelled as Ac_1 (R_f 0.16), Ac_2 (R_f 0.17), Ac_3 (R_f 0.26), Ac_4 (R_f 0.35), Ac_5 (R_f 0.44), Ac_6 (R_f 0.51), Ac_7 (R_f 0.70), Ac_8 (R_f 0.77) and Ac_9 (R_f 0.78).

Separation of Biflavonoid Mixture-Preparative thin-layer-chromatography

Using a thin layer applicator, glass plates (20 x 20 cm) were coated with a well stirred suspension of Si-gel G (E. Merck 35 gm, 70 ml water) to give a layer, approximately, 0.5 mm in thickness. After drying for 2 hrs at room temperature, the plates were activated at 110-120° for 1 hr. The crude solid was dissolved in pyridine and was applied with the help of applicator. The plates were mounted on a stainless steel frame and placed in Desaga glass chamber 10 x 22 x 21 cm containing 300 ml of developer (BPF 36:9:5). When the solvent front travelled 15 cm from the starting line the development was interrupted and the plates were dried at room temperature. The positions of the bands were marked in U.V. light. The marked zones were scraped and eluted in separate columns with dry acetone and pyridine, the solvent was recovered till the eluent was reduced to 20-30 ml. The addition of water and HCl yielded the yellow precipitate in each case which was filtered, washed with water and dried. Homogeniety of the individual components was checked by TLC. The components were labelled as Ac_1-Ac_9.

Characterization

The complexities of different fractions were studied by TLC examination of their fully methylated products.

Methylation of Ac_1

Ac_1, minor constituent, was methylated using appropriate quantity of dimethyl sulfate and freshly ignited potassium carbonate in dry acetone. It was refluxed on water bath for about 6 hrs. A small portion of the reaction mixture was taken out and tested for alcoholic ferric chloride reaction. Refluxing continued untill it gave a negative ferric chloride test. It was then filtered and evaporated to dryness. The yellow solid mass left behind was treated with petroleum ether and then dissolved in chloroform. The chloroform solution was washed with water, dried over anhydrous sodium sulfate and concentrated to give a crude solid. The methylated mixture, on TLC examination, showed the presence of agathisflavone hexamethylether (R_f 0.45) and robustaflavone hexamethylether (R_f 0.49) which were identified by comparison with authentic samples and characteristic shades in U.V. light.

Ac_2

It was methylated by the procedure mentioned in the case of Ac_1. The methylated product was examined on thin

layer chromatography using silica gel as adsorbent and Benzene:Pyridine:Formic acid (36:9:5) as developer and compared with the authentic samples (R_f values and characteristic fluorescence in U.V. light). TLC of the methylated product revealed the presence of amentoflavone hexamethylether (R_f 0.40) and cupressuflavone hexamethylether (R_f 0.43).

Ac_3

It was methylated as in the case of Ac_1. TLC of the methylated product was performed on silica gel using Benzene:Pyridine:Formic (36:9:5) as developer and compared with authentic samples (R_f values and characteristic shades in U.V. light). TLC of the methylated product revealed the presence of agathisflavone hexamethylether (R_f 0.45) and robustaflavone hexamethylether (R_f 0.49).

Ac_4

The methylated product, obtained by the methylation of Ac_4 using the procedure similar to Ac_1, was examined on thin layer chromatography using silica gel as adsorbent and Benzene:Pyridine:Formic acid (36:9:5) as developer and compared with authentic samples. TLC of the methylated product indicated the presence of cupressuflavone hexamethylether (R_f 0.43), amentoflavone hexamethylether (R_f 0.40) and hinokiflavone pentamethylether (R_f 0.48).

Ac_5

This fraction was methylated as in the case of Ac_1. TLC of the methylated product was performed on silica-gel using Benzene:Pyridine:Formic acid (36:9:5) as developer and compared with authentic samples (R_f values and characteristic shades in U.V. light) which revealed the presence of only agathisflavone hexamethylether (R_f 0.45).

Ac_6

It was methylated by the procedure mentioned in the case of Ac_1. The methylated product was examined on thin layer chromatography using silica-gel as adsorbent and Benzene: Pyridine:Formic acid (36:9:5) as developer and compared with the authentic samples. TLC of the methylated product revealed the presence of amentoflavone hexamethylether (R_f 0.40) and cupressuflavone hexamethylether (R_f 0.43).

Ac_7

This fraction was methylated as in the case of Ac_1. The TLC of methylated product was performed on silica gel using benzene:pyridine:formic acid (36:9:5) as developer and compared with authentic samples (R_f values and characteristic fluorescence in U.V. light). Amentoflavone hexamethylether (R_f 0.40) and cupressuflavone hexamethylether (R_f 0.43) were identified in the permethylated product of Ac_7.

Ac_8

It was methylated by the procedure mentioned as in the case of Ac_1. The methylated product was examined on thin layer chromatography using silica-gel as adsorbent and Benzene:Pyridine:Formic Acid (36:9:5) as developer and compared with authentic samples. TLC of the methylated product revealed the presence of only amentoflavone hexamethylether (R_f 0.40).

Ac_9

It was methylated as in the case of Ac_1. The TLC of methylated product was performed on silica-gel using Benzene:Pyridine:Formic acid (36:9:5) as developer and compared with authentic sample. TLC of methylated product indicated the presence of only cupressuflavone hexamethylether (R_f 0.43).

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